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A Study of Several Strains of Pleomorphic Streptococci.

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KLEIN AND GORDON[1] isolated a polymorphic streptococcus from a series of scarlet fever cases and called it *Streptococcus scarlatina*. They considered it identical with the *Streptococcus conglomeratus* of Kurth.[2] Klein held that this streptococcus is causally related to scarlet fever in man and is wholly distinct from *Streptococcus pyogenes*. Gordon believed that both *Streptococcus pyogenes* and *Streptococcus conglomeratus* may play a part in the causation of scarlet fever, but that *Streptococcus conglomeratus* is the more important of the two, and that it occupies a position in the bacteriological kingdom between *Streptococcus pyogenes* and *Bacillus diphtheria*.

Winslow[3] mentions the fact that cocci isolated freshly from the mouth are particularly apt to show successive pairs of flattened cells and occasionally exhibit elongated rod-like cells, and that large cells sometimes appear in chains of smaller cells. This is probably a generally observed fact, although practically no mention is made of it in the literature.

Newman[4] emphasizes an observation of Gordon that frequently the streptococcus may morphologically resemble the Klebs-Loeffler bacillus.

Jean Broadhurst[5] has more recently observed large cells appearing in sugar-broth cultures of streptococci, and remarks upon the absence of reference to such variation in the literature.

Behring[6] investigated the *Streptococcus longus* and recognized four groups. Under group three he places Kurth's *Streptococcus conglomeratus* as one yielding or producing scaly flocks of sediment. Andrew and Horder[7] have classified as *Streptococcus anginosus* a pathogenic long-chained form, allied in other respects to *Streptococcus salivarius*, and bearing to it much the same relation which

Streptococcus pyogenes bears to *Streptococcus mitis*. They mention it as occurring in cases of scarlatinal and other forms of sore throat. It produces a flocculent deposit in broth, clots milk, reduces neutral red, forms acid in saccharose, lactose, and raffinose.

The enterococcus of Thiercelin, Escherich, Beeson[8] and others is a pleomorphic streptococcus that varies from the streptococcus of Gordon in its growth in broth, milk and on agar.

The object of this paper is to report upon a pleomorphic strain of the streptococcus isolated from chronically enlarged cervical lymph glands of a patient otherwise apparently in good health, and to compare this with eight other more or less markedly pleomorphic streptococci isolated from five cases of tonsilitis, two cases of bronchitis, and one case of infection on back of hand and with *Streptococcus pyogenes* and *Bacillus diphtheria*. The basis of comparison is upon morphology, staining reactions, cultural characteristics, action on carbohydrates according to Andrew's and Horder's classification, and complement fixation tests.

In November, 1912, a local physician requested a bacteriological examination of material from the cervical lymph glands of a patient with the following history: Male, age 26; had noticed twenty-six months previously the enlargement of the cervical lymph glands on the right side of the neck. Nineteen months later he attended a hospital clinic, drainage was established, and the case observed at intervals by the attending physician for seven months. During this time the glands continued to enlarge. At the end of seven months the patient went to his family physician, who suggested the bacteriological examination. No acid-fast organisms were found, but a pure culture of a marked pleomorphic streptococcus was obtained both from the glands and from the tonsils. At this time the glands on both sides were involved; the axillary and inguinal lymph glands were shotty. The physician advised a tonsillectomy, but the patient wanted a vaccine. Accordingly a vaccine was prepared. Three weeks after the administration of the first dose the swelling had disappeared and the patient was discharged. Three months afterward there seemed to be the beginning of a recurrence, a tonsillectomy was performed, and the patient has remained well to date.

The organism isolated had the following characteristics, which, in so far as morphology, staining reactions and general cultural characteristics are concerned, seemed to be identical with those of *Streptococcus scarlatina* of Klein and Gordon and the *Streptococcus*

conglomeratus of Kurth. It apparently varied somewhat from these in its action on some of the carbohydrates and glucosides and was not isolated from a scarlet fever patient.

MORPHOLOGY. A pleomorphic streptococcus, showing irregularities in size and shape of elements, every transition between coccus form and bacillus form. This includes wedge shape, spindle shapes, oval forms, club shapes, and tendency towards filaments in agar, gelatin and modified Loeffler's blood serum. In hanging drops there were conglomerate coherent masses.

STAINING REACTIONS. Stained readily by the ordinary aniline stains, intensely Gram positive and non-acid-fast. Metachromatic granules were not observed.

CULTURAL CHARACTERISTICS. Agar slant, 37° C., three types of colonies after twenty-four hours: (*a*) gray, granular, irregularly outlined colonies; (*b*) colonies quite similar but showing a confluent appearance; (*c*) colonies evidently younger and smaller, which have a fine frilling of chains around a more dense compact confluent center.

Gelatin, 20° C., non-liquefying.

Bouillon, 37° C., clear, coherent sediment in bottom of tube.

Litmus milk, 37° C., twenty-four hours, coagulated rapidly, usually with quite a solid curd, lower half of tube white, while upper half was pink.

Carbohydrates and glucosides. Dextrose, lactose, saccharose and raffinose acidified, while inulin, salicin and mannite were not attacked. Neutral red showed some reduction.

Russell's medium, acidified. When, instead of a central stab, a stab was made next to the glass, the growth assumed a typical skyrocket appearance, spreading out near the bottom of the tube. The lower part of the tube was decolorized.

POSITION ACCORDING TO ANDREW'S AND HORDER'S CLASSIFICATION. In the following table a comparison of the action of this pleomorphic streptococcus with other streptococci is given according to their action on various carbohydrates, glucosides and neutral red.

	Milk	Neutral red	Saccharose	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite
Pleomorphic streptococcus	+	+*	+	+	+	−	−	.....	−
Strep. equinus	−	−	+	−	−	−	+	+	−
Strep. mitis	A	−	+	+	−	−	+	−	−
Strep. pyogenes	A	−	+	+	−	−	+	−	−
Strep. salivarius	+	+	+	+	+	−	−	−	−
Strep. anginosus	+	+	+	+	+	−	−	−	−
Strep. fecalis	+	+	+	+	−	−	+	+	+
Strep. pneumonia	A	−	+	+	+	+	−	−	−

NOTE.—In the above table in the column for milk (+) signifies acid with clotting, (A) acid without clotting, (−) no apparent action. For neutral red (+) signifies reduction, (+*) partial reduction, (−) no action. Otherwise (+) signifies acid to litmus, (−) no action or alkaline.

Winslow[9] seems to think that the *Streptococcus scarlatina* described by Gordon and the *Streptococcus conglomeratus* of Kurth were the same as *Streptococcus pyogenes*. He bases his statement upon the further studies of Gordon upon this group from the standpoint of their fermentation reactions. It will be observed that the pleomorphic streptococcus described in this paper would, according to Andrew's and Horder's classification, correspond to *Streptococcus anginosis*.

Since this organism was isolated the author has made slides and cultures from 365 cases of tonsillitis occurring among students and faculty of the University and from other cases in the city, from several cases of bronchitis and one case of hand infection. In addition, fifty cultures and smears were made from the throats of apparently normal individuals. Streptococci with large cells and with rod-like cells were found very commonly in the cases of tonsillitis and frequently in bronchitis. Only a very few were obtained from the normal throats. These were from individuals subject to attacks of tonsillitis as a rule. Five strains were obtained from cases of tonsillitis, two from cases of bronchitis, and one from infection on back of hand of a member of the faculty, all of which showed marked tendency toward persistent involution and produced more marked conglomerate masses in the bottom of broth tubes. They correspond very closely in morphology, cultural characteristics and tinctorial reactions with the organism described above. From four of these cases fresh material was obtained and isolations of club-shaped organisms and chains with enlarged cells were made by Barber's pipette method for single

cell isolation. The individual organisms were inoculated into the moisture of condensation of agar slants, and after several hours the water was permitted to cover the surface of the slant. In this way growth was obtained. The author was unable to get very large individual coccus forms to grow satisfactorily, although Dr. F. Hecker has privately reported success. The resulting cultures from the above isolations showed as marked pleomorphic variations as the other cultures. As a rule, successive transfers in dextrose broth would yield uniform chains in time, but involution forms reappeared when transfers were made to solid media, especially if partially desiccated media were used. Very frequently beautiful chains could be obtained from the deeper part of the stab in Russell's medium when the stab was made next to the glass, while involution forms predominated upon the surface of the slant.

The *Streptococcus pyogenes*, and in fact the rest of the streptococcus group, is usually described as chains of cocci of comparatively uniform size, but varying in length of chain, pathogenicity, action on litmus milk, various carbohydrates, glucosides and neutral red.

Much work has been done which suggests that perhaps there are not so many different streptococci as has been generally thought, but that one or at most a few organisms make up the group and that these are very plastic and subject to much variation. Heineman[10] and others have concluded that *Streptococcus pyogenes* and *Streptococcus lacticus* are perhaps one and the same. Rosenow[11] has reported remarkably induced mutations in streptococci. He has converted hemolytic streptococci from many sources, such as erysipelas, scarlet fever, puerperal fever, arthritis, tonsillitis, milk, etc., into *Streptococcus viridans*, *Streptococcus mucosus*, and typical pseudopneumococci; *Streptococcus viridans* into *Streptococcus mucosus*, *Streptococcus hemolyticus* and *Streptococcus rheumaticus; Streptococcus mucosus* into *Streptococcus viridans* and *Streptococcus hemolyticus; Streptococcus rheumaticus* into *Streptococcus viridans* and *pneumococci*. In this work he made use of pure cultures isolated by Barber's pipette method for single cell isolation. Billings and Rosenow[12] record quite a remarkable observation of bacterial mutation from a bacillus to a coccus form. They report colonies on dextrose agar showing only bacillus forms yielded in subculture; a staphlococcus in pure cultures; and forms of the bacillus, either pure or in a mixture, anerobically on the same media.

Dr. Victor C. Vaughn,[13] citing the collected studies of Eisenberg on "Mutation in Bacteria," gives a splendid brief discussion of the subject. In conclusion he says: "Evidence of mutation in bacteria might be multiplied many times. It is shown in changes of form, in capsule formation, in production of spores, in alteration of virulence, etc., but I think that I have collected enough data to conclusively show that in bacteria acquired characters are in part at least inheritable."

The tendency toward pleomorphism was quite persistent for the streptococci reported upon in this paper. After seven months cultivation there was a tendency toward uniform chain formation, but decided involution forms would occur. The variation in morphology of streptococci in liquid media, such as mannite broth, saccharose broth, etc., reported by Jean Broadhurst[14] was much more temporary but quite interesting. She says: "The morphology of the individual cell may be likewise affected. In media not utilized, some of the chains are usually of full or increased length, and there is usually a small proportion of swollen organisms (rounded and elliptical) either in short chains or interposed here and there in chains composed mainly of normal organisms. When the media are utilized, there is, besides this swelling, a distinct tendency to abnormal shapes . . . Often, however, more varied forms are seen—organisms which may be actually pear-shaped, club-shaped or obtusely diamond shaped. These changes are most marked in mannite, though they may occur in other media. . . . . A normal appearance is effected at once by transplanting to plain broth." Jean Broadhurst also made another interesting observation, which was that a strain of streptococcus producing no change in litmus milk acquired the ability of producing acid and of coagulating the milk by being put into a capsule and passed through the intestinal tract of a dog.

Repeated attempts have been made without success to control the pleomorphism of these organisms. In view of the observation that involution forms appeared quite frequently upon partly desiccated solid media, it was thought that perhaps differences in osmotic pressures might be responsible for the variations. Accordingly bouillon containing different concentrations of dextrose were tried, as were also different concentrations of NaCl, but the involution could not be controlled or increased in slightly involuting strains. Then culture media containing different concentrations of HCl and NaOH were tried, with unsatisfactory results. Since in the first organism isolated it was possible to get

uniform chain formation under partial anerobic conditions, it was decided to try increasing the oxygen tension slightly above atmospheric and see if this had any effect upon involution. This gave unsatisfactory results, as well as an experiment in which the CO_2 content of the atmosphere in contact with the culture media was varied. These negative results naturally do not mean that these involutions can not be controlled, as no doubt that time and patience or good fortune would yield positive results. I might say, however, that some of my work on another problem has suggested that perhaps the pleomorphism on the partially desiccated agar was due to the salts that crystallized out on the surface of the agar. It is hoped that this may be checked up more fully in the future.

Even if involution forms could be produced at will under one set of conditions, it would not necessarily follow that these were the conditions holding forth in the body under which pleomorphism occurred or persisted. The variation of the morphology of these organisms from typical streptococci have been termed involution forms, largely because (1) these organisms seem to prefer, as a rule, to grow and act as streptococci under artificial conditions of laboratory cultures; (2) the streptococci as a group is known to be very plastic, mutations occurring in many different ways; (3) similar though more transient variations in morphology have been observed to occur in pure cultures of streptococci; (4) since sufficient evidence is not apparent to change the classification given them by Kurth, Gordon and Klein.

The similarity to *B. diphtheria* noted by Gordon and emphasized by Newman is at times quite striking. Not enough strains of these organisms were studied to warrant a statement that none might contain metachromatic granules, since it is a well-known fact, as emphasized by Graham Smith,[15] that not all strains of virulent *B. diphtheria* have metachromatic granules. None were observed in the cultures studied. I know that frequently these pleomorphic streptococci may be mistaken for *B. diphtheria* when smear preparations only are examined. A bacteriologist experienced in diphtheria examinations would not make the mistakes, but when one realizes that many relatively inexperienced individuals make throat examinations, either for themselves or others, it is quite possible for errors to be made. These errors would be upon the positive side and might or might not be objectionable, depending upon the tolerance of the individual for the foreign protein, any benefit derived from it, the expense incident thereto,

and the suffering entailed. Four of the eight severe cases of tonsillitis were given 10,000 units of antitoxin and made a rapid recovery. Of the other four cases, two cleared up in time though not nearly so quickly, and two developed ear infections and responded to vaccine treatment. All were suspected diphtheria cases. It might be said, in the first place, that the number of cases is too few for the results to be of value; second, that in the four cases receiving antitoxin, they would have made a quick recovery anyway; third, that in these four there were foci of infection with *B. diphtheria* in the trachæ (nose and throat examinations were made); fourth, that the diphtheria antitoxin was beneficial because of a relationship between the diphtheria group and this group (these organisms were not toxin producers in vitro); fifth, the benefit resulted from nonspecific protein therapy as emphasized by Muller, Weichardt, Miller and others for many infections. If the marked improvement resulted from the injection of the antitoxin, I would rather incline to the last explanation, perhaps, until a more definite relationship is proven between *B. diphtheria* and the streptococci than I have found. It is, however, true that Denny, Corbett and others have described strains of *B. diphtheria* that they call streptococcal forms, and the pleomorphic nature of *B. diphtheria* is well known. From the standpoint of morphology *B. diphtheria* has been frequently reported as showing involution forms similar to these pleomorphic streptococci. G. S. Graham-Smith[16] describes the involution forms of *B. diphtheria* as follows: "After prolonged growth on a suitable medium, or more quickly on an unsuitable medium, diphtheria bacilli become considerably altered in shape. Their appearance becomes more irregular and very large forms are frequently encountered. Many become greatly swollen at the ends and develop enormous club-shaped masses which stain deeply, while the rest of the bacillus may stain badly or have irregular patches of deeply stained protoplasm in it. Others become pear-shaped or globular, while some retain their general shape but become thicker throughout. Others again show large globules at their ends, while the rest of the rod appears as a faintly stained line. Specimens which take up the stain very badly are common. Some bacilli may be represented by small round masses like cocci, or by a chain of such masses, when they look like streptococci. In fact, under such conditions every variety of shape and form and staining capacity may be met with." The cultural characteristics of *B. diphtheria* are well known. It is generally considered that the organism ferments dextrose but not

lactose. Graham-Smith,[17] however, reports numerous strains of virulent diphtheria bacilli which produced acid with coagulation of Hiss's serum water medium containing lactose. The diphtheria bacillus grown in dextrose broth gives a typical streptococcus effect, clear broth with flocculent precipitate settling down. Macroscopically the colonies on agar are quite similar to normal streptococcus colonies. Its action in neutral red is variable. It must be remembered that one of the strong objections to classification of any organisms according to fermentation reactions is because of the fluctuating variability that one frequently meets with. I did not think that the pleomorphic streptococci isolated were identical with *B. diphtheria*, but I was interested in the apparent and suggested relationship between the streptococcus group and the diphtheria group, as suggested by Gordon and others. I next decided to compare the several strains of pleomorphic streptococci with each other and with *B. diphtheria* and *Streptococcus pyogenes* by means of the complement fixation test. I wanted to include a known culture of *Streptococcus anginosis*, but was unable to obtain it at the time. This work was then conducted as follows:

ANTIGEN. Two kinds of antigen were employed. In both cases the organisms were grown in plain agar. For one of the antigens suspension of the growth in isotonic salt solution was used. For the other type of antigen the growth was teased off in distilled water and suspension allowed to autolyze and an equal volume of double-strength salt solution added.

HEMOLYTIC SYSTEM. Human red blood cells and the corresponding hemolytic amboceptor.

TECHNIQUE. The technique, barring of course the differences in antigens, was similar to Noguchi's modification of the Wassermann reaction. As a preliminary to this work all of the animals were checked up for normal complement-fixing antibodies with each of the above antigens, and all were negative.

Following vaccination with the respective strains of organisms, the results showed cross-fixation for all of the strains of the pleomorphic streptococci, but no fixation when any of these pleomorphic streptococci were used as antigen with anti-*Streptococci pyogenes* immune sera or *B. diphtheria* immune sera, nor was the converse true. Neither was there any cross-fixation between *B. diphtheria* and *Streptococcus pyogenes*, although these antigens gave 4 plus reactions with their respective immune sera. In other

words, no relationship was apparent or suggested between these pleomorphic streptococci and *B. diphtheria* and *Streptococcus pyogenes* as suggested by Gordon. It might well be that a relationship could exist and the complement fixation test be unreliable. Meyer,[19] Aronson,[20] Kerner,[21] while not working with complement fixation test, decided that immune sera gave variable results and were not consistent. Some recent work on the streptococcus group in press is quite desirable. The complement fixation test has not been a uniform and decided success as a means of differentiation, but nevertheless is of great value in many instances.

Since this paper was finished two papers by Mellon on the diphtheroid group of organisms have appeared. He reports upon a pleomorphic organism isolated from the lungs which shows all variations from bacillary form to a streptococcus form. In morphology and many cultural reactions his diphtheroid is similar to the pleomorphic streptococcus I have been working with. Both are pleomorphic, Gram-positive, nonmotile organisms giving a clear broth with heavy sediment, coagulate milk, produce acid in dextrose, lactose, sucrose. The diphtheroid organism produced acid in salicin and inulin, whereas the pleomorphic streptococcus I am reporting on failed to do so. Mellon states that in broth cultures at one stage of development there are numerous bipolar bacilli with large granules. From this I presume that metachromatic granules were observed. I did not observe these occurring in the pleomorphic streptococcus studied. Doctor Mellon has placed his organism among the diphtheroids because of immunological, cultural and morphological reasons; however, he says that "unless great care is used in handling the recently recovered bacillary form, it will almost immediately revert to the diplococcus form when transplanted even to solid media." His experimental work and mine, made in attempts to control the pleomorphism, give largely negative results.

I should say that we were both working with closely related if not identical organisms. In his papers he suggests the immunological relationship between his organism and the group of diphtheroids, of *B. diphtheria* and the streptococci. As a result of complement fixation work, I found no relationship between the pleomorphic organisms I was studying and *B. diphtheria* or *Streptococcus pyogenes*.

SUMMARY AND CONCLUSIONS.

1. That a pleomorphic organism quite similar to if not identical with *Streptococcus scarlatina* of Gordon and *Streptococcus conglomeratus* of Kurth was isolated by me from chronically enlarged cervical lymph glands of an individual having no history of scarlet fever. This organism showed all transitions between bacillary form and chains of diplococci.

2. That a vaccine was apparently beneficial, and that since tonsillectomy no recurrence has taken place. The tendency for recurrence after three months is another example of the transient immunity resulting from streptococcus vaccination. It is a well-known fact that streptococcus infections do not confer a lasting immunity. This is one thing that should give us pause in ascribing to streptococci the primary cause of such diseases as scarlet fever and measles where a comparatively lasting immunity results.

3. That a similar organism was isolated from a hand infection, from two cases of bronchitis, and from five out of 365 cases of tonsillitis. Frequently pleomorphic streptococci showing other colony types on agar and Loeffler's blood serum were also observed in tonsillitis cases.

4. That some of the involution forms of this streptococcus might be mistaken for *B. diphtheria* by other than a well-trained observer. This is of importance in view of the many relatively inexperienced individuals doing laboratory work either for themselves or as a commercial proposition. It is of added interest in view of the observations of Gordon and Newman, suggesting a relationship between the streptococcus group and the diphtheria group. This has more recently been emphasized by Mellon.

5. That during the diphtheria epidemic in Kansas City in 1914 a number of patients showing only pleomorphic streptococci in nose and throat smears and cultures, nevertheless recovered rapidly following administration of 10,000 units of antitoxin. Similar results were obtained in a small series of cases in Lawrence. This may or may not mean anything. If the apparent beneficial results were real and not a coincidence, and if no unfound foci of diphtheria existed, it might not indicate a relationship between these organisms and *B. diphtheria*, but could be another example of nonspecific protein therapy as reported by Muller, Miller, Weichardt and others.

6. That according to Andrew and Horder's classification the pleomorphic organism I am reporting upon appeared to be the same as *Streptococcus anginosus*.

7. It ultimately seemed to prefer the streptococcus (chains of diplococci) morphology.

8. Complement fixation tests showed no normal complement fixing bodies, in the normal rabbits used, for any of the pleomorphic streptococci, *Streptococcus pyogenes* or *B. diphtheria.*

9. Complement fixation with respective immune sera showed no relationship between the pleomorphic streptococcus and *B. diphtheria.* This, however, does not necessarily prove no relationship.

10. While attempts to control the pleomorphism were largely negative, I have some results which suggest that the salts which crystallized out on the agar might be a factor in producing involution forms on the desiccated agar.

11. I feel that a healthy skepticism is well worth while in all work involving mutation, since errors in technique can lead to very erroneous conclusions. The work of Mellon seems to be thoroughly and carefully done and is worthy of consideration. His results, even more than mine, point to an intermediate group between the streptococci and diphtheria.

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